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Various growth factor receptor pathways promote human breast tumorigenesis of hormone-independent tumors. The nuclear receptor coactivator AIB1 (amplified in breast cancer 1) can be phosphorylated and regulated by growth factor-induced signaling pathways such as MAP kinase and IkB kinase. Our lab has found a splice variant of AIB1, called delta exon3 AIB1, which has a higher co-activating ability than the full-length protein. This study determined the ability of delta exon3 splice variant compared with AIB1 in potentiating growth factor signaling and to determine the mechanism of this potentiation using a growth factor responsive promoter.

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Table of Contents

INTRODUCTION

AIB1 (Amplified in Breast Cancer 1) is a member of the p160 family of steroid receptor coactivators. AIB1 is located on chromosome 20q and its gene is amplified in 5-10% of primary breast cancers (1). AIB1 mRNA is overexpressed in 60% of breast tumors (2) and its increased protein levels have been correlated with tumor size, p53 status and HER2/neu expression (3). High expression of AIB1 in breast tumors with high HER2/neu expression in patients that received tamoxifen therapy had the worst disease free outcome compared to all other patients (4, 5). AIB1 has been increasingly associated with function and regulation by growth factor non-steroid hormone induced signaling. Disruption of p/CIP, the mouse homolog of AIB1, results in a pleiotrophic phenotype including reduced female reproductive function and blunted mammary gland development in mice (6, 7). Overexpression of AIB1 in transgenic mice resulted in increased mammary gland proliferation and up regulation and activation of pro-proliferative markers, cyclin D1 and IGF-1 receptor (8). AIB1 has also been demonstrated to be a down stream target for phosphorylation by MAP kinase (9), IkappaB kinase (10) and is involved in regulating AKT kinase expression levels (11). These data reinforce the role of AIB1 in wide-ranging effects on tumor growth that is independent of steroid receptor function.

BODY

The research accomplishments in this report include more data to address **Task 1** in the approved **Statement of Work** in the original grant application, DAMD17-02-1-0394. As stated in the April 2004 report, two of the key research accomplishments were the discovery that AIB1 and Δexon3 AIB1 isoform is critical for IGF-1 stimulated anchorage independent growth and both AIB1 and Δexon3 AIB1 isoform are necessary for maintaining the expression of the protein Cyclin D1.

To further determine other genes that are important in maintaining and enhancing anchorage independent growth in which AIB1 and Δexon3 AIB1 isoform are rate limiting for, a cDNA microarray analysis was performed. A cDNA microarray (also known as a gene array or DNA chip) analysis was utilized because it allows one to determine the expression of thousands of genes at the same time. The Affymetrix U133A human gene chip that represents 33,568 transcripts was used to evaluate gene expression profiles of the MCF-7 breast cancer cell line that was treated either with control (scrambled) or AIB1/Δexon3 AIB1 isoform siRNA for 48 hours. Total mRNA was harvested from the siRNA transfected MCF-7 cells and used to synthesize double stranded cDNA. T7 primers were used to prime the first strand cDNA synthesis. Synthesis of biotin labeled complementary RNA (cRNA) was synthesized from the double stranded cDNA and used to hybridize the Affymetrix U133A human genome chip. MCF-7 cells either transfected with the control or AIB1 siRNA were analyzed using four U133A chips. For each of the eight arrays, measurements from 22,283 genes were obtained. Two sample *t* tests were carried out to compare the expression intensity between AIB1 high (control siRNA transfected) and AIB1 low (AIB1 siRNA transfected) arrays for each of the genes found on the four arrays per group using the randomized variance model statistical analysis. A total of 124 genes demonstrated a statistically significant difference between AIB1 high and AIB1 low at $P \leq 0.0025$ from the univariate test. The 124 genes included genes that were both up and down regulated by AIB1 and Δexon3 AIB1 isoform. The genes were also ranked according to their *P* value.

The 124 genes were separated into tables based on function and involvement in biological processes into the following categories: apoptosis, cell/cycle, proliferation, cytoskeleton modifying, GTP binding, metabolism/biosynthesis, protein modification, secreted factors, transcription, transport, and genes with various or unknown functions (miscellaneous). Interestingly, several

genes that are known to be critical for cell cycle regulation, anoikis, and apoptosis, notably Cyclin D1, Bcl-2, MAPK (ERK1/2), were highly dependent on AIB1 and Δexon3 AIB1 isoform levels for their sustained expression (highlighted ** in Tables 1A-J).

To assess whether altered mRNA expression as detected by the cDNA microarray analysis due to AIB1 and Δexon3 AIB1 isoform reduction was also reflected at the protein level, I performed a series of Western blot analysis for those proteins known to be crucial for cell growth and survival. The protein levels of Cyclin D1, Bcl-2 and ERK2 (but not ERK1) were reduced (90%, 40% and 40% respectively) by lowering the AIB1 levels by >90% in attached MCF-7 cells (Fig. 1). AIB1 siRNA reductions in expression of these genes were also observed in the presence of IGF-1 (Fig. 1). IGF-1 clearly induced the expression of Cyclin D1 (4-fold) and caused a smaller 1.7 fold increase in the protein expression of ERK2 (Fig. 1). I examined the effect cell detachment/suspension on the expression of the cyclin D1, bcl-2 and ERK2. Cell suspension was achieved by coating the dishes with poly-HEMA, a bio-gel that prevents cells from attaching to the cell culture plate. The basal levels of both Cyclin D1 and Bcl-2 were increased under anchorage independent conditions (Fig. 1) and IGF-1 did not increase these levels any further (Fig. 1). Overall it is clear that in basal or IGF-1 treated conditions, in cells attached or in suspension, targeting of AIB1 was effective in producing decreases in Cyclin D1, Bcl-2 and ERK2 protein levels. Maintenance of the expression of these genes by AIB1 could be involved in both basal and IGF-1 induced growth responses in MCF-7 cells.

Table 1a

"apoptosis"	*Probe set*	*mean expression for AIB1 high*	*mean expression for AIB1 low*	*Fold (AIB high/AIB low)*	*Up (+) or down (-) regulated by AIB1*	*rank of P value of the randomized variance test*	*P value of the randomized variance test*
p53-induced protein PIGPC-1 [THW, KCP1, PIGPC1]	217744_s_at	571.677	1195.691	0.478	-	3	0.0000321
***Bcl-2*	*207005_s_at*	*104.988*	*26.786*	*3.92*	+	*101*	*0.0018496*

Table 1b

"cell cycle/proliferation"	*Probe set*	*mean expression for AIB1 high*	*mean expression for AIB1 low*	*Fold (AIB high/AIB low)*	*Up (+) or down (-) regulated by AIB1*	*rank of P value of the randomized variance test*	*P value of the randomized variance test*
***cyclin D1*	*208712_at*	*3262.634*	*1292.907*	*2.523*	+	*5*	*0.0000426*
***MAPK-1*	*212271_at*	*1279.667*	*569.648*	*2.246*	+	*20*	*0.0001316*
HMBA-inducible	202814_s_at	528.328	934.206	0.566	-	32	0.0002883
insulin induced gene 1	201626_at	1384.879	2681.165	0.517	-	88	0.0013424
serum/ glucocorticoid regulated kinase-like	220038_at	187.597	84.055	2.232	+	89	0.001394
WNT inhibitory factor 1	204712_at	21.107	63.23	0.334	-	92	0.0014629
platelet-derived growth factor receptor-like	205226_at	88.627	137.983	0.642	-	107	0.002008
c-src tyrosine kinase	202329_at	619.421	1025.285	0.604	-	108	0.0020142

Table 1c

"cytoskeleton modifying"	*Probe set*	*mean expression for AIB1 high*	*mean expression for AIB1 low*	*Fold (AIB high/AIB low)*	*Up (+) or down (-) regulated by AIB1*	*rank of P value of the randomized variance test*	*P value of the randomized variance test*
filamin B	208613_s_at	823.305	389.677	2.113	+	33	0.0002931
PHACTR2 phosphotase actin	204049_s_at	588.212	1310.993	0.449	-	46	0.0005077
ectonucleoside triphosphoate diphosphohydrolase 1	207691_x_at	145.162	271.393	0.535	-	51	0.0005858
keratin 8	209008_x_at	19559.105	10904.508	1.794	+	67	0.0008456
coiled-coil protein BICD2	213154_s_at	356.548	616.454	0.578	-	99	0.0018189
caldesmon 1	205525_at	29.357	46.41	0.633	-	116	0.0022346
myristoylated alanine-rich protein kinase C substrate	201669_s_at	656.271	1391.009	0.472	-	81	0.001137

Table 1d

"GTP binding"	*Probe set*	*mean expression for AIB1 high*	*mean expression for AIB1 low*	*Fold (AIB high/AIB low)*	*Up (+) or down (-) regulated by AIB1*	*rank of P value of the randomized variance test*	*P value of the randomized variance test*
RAB, member of RAS oncogene family-like 4	205037_at	274.876	654.179	0.42	-	1	0.00002
RAB27B, member RAS oncogene family	207017_at	44.51	111.832	0.398	-	7	0.0000511
RAB 15, member RAS oncogene family	59697_at	1070.718	574.105	1.865	+	49	0.0005535
Cdc42 guanine nucleotide exchange factor (GEF) 9	203264_s_at	41.278	101.05	0.408	-	54	0.0006257
phosphoenolpyruvate carboxykinase 2 (mitochondrial)	202847_at	897.913	1530.352	0.587	-	103	0.0018568
Tara-like protein	210276_s_at	268.181	592.816	0.452	-	120	0.002376

Table 1e

"metabolism/biosynthesis"	*Probe set*	*mean expression for AIB1 high*	*mean expression for AIB1 low*	*Fold (AIB high/AIB low)*	*Up (+) or down (-) regulated by AIB1*	*rank of P value of the randomized variance test*	*P value of the randomized variance test*
malic enzyme 1, NADP(+)-dependent, cytosolic	204058_at	440.668	1044.197	0.422	-	2	0.0000242
3'-phosphoadenosine 5'-phosphosulfate synthase 2	203058_s_at	81.223	179.569	0.452	-	13	0.0001073
3'-phosphoadenosine 5'-phosphosulfate synthase 2	203060_s_at	125.006	297.243	0.421	-	23	0.0001616
dehydrogenase/reductase (SDR family) member 2 (DHRS2)	214079_at	1694.251	329.078	5.148	+	30	0.0001957
molybdenum cofactor synthesis 2	218212_s_at	1431.594	624.03	2.294	+	31	0.0002208
kynureninase (L-lynurenine hydrolase)	217388_s_at	277.552	626.48	0.443	-	35	0.0003291
methionyl aminopeptidase 2	202015_x_at	27.966	59.198	0.472	-	37	0.0003691
glyoxalase I	200681_at	5937.301	2780.38	2.135	+	38	0.0003918
hydroxy prostaglandin dehyrogenase 15-(NAD)	211548_s_at	244.116	100.061	2.44	+	44	0.0004619
dodecenoyl-Coenzyme A delta isomerase (3,2 trans-enoyl-Coenzyme A isomerase)	209759_s_at	653.268	1380.144	0.473	-	48	0.0005196
solute carrier family 7 (cationic amino acid transporter, y_system), member 5	201195_s_at	6410.213	2766.12	2.317	+	58	0.0007245
family with sequence similarity 16, member A, X linked	203974_at	933.932	388.651	2.403	+	61	0.0007798
ATP-binding cassette, sub-family C	208161_s_at	253.505	697.4	0.364	-	70	0.0009091
deiodinase, iodothyronine, type I	206457_s_at	72.876	139.655	0.522	-	72	0.0009778
insulin induced gene 1	201626_at	1384.879	2681.165	0.517	-	88	0.0013424
alpha glucosidase II alpha subunit	211934_x_at	853.545	1802.795	0.473	-	98	0.001746
UDP-GlcNAc:betaGal beta-1,3,-N-acetylglucosaminoyltransferase 6	203188_at	875.021	555.977	1.574	+	106	0.0019878
hydroxy prostaglandin dehyrogenase 15-(NAD)	211549_s_at	150.119	85.033	1.765	+	111	0.0021083
galactokinase 2	205219_s_at	240.849	388.952	0.619	-	114	0.0022089
hydroxy prostaglandin dehyrogenase 15-(NAD)	203913_s_at	255.449	117.952	2.166	+	117	0.0022542

Table 1f

"protein modification"	*Probe set*	*mean expression for AIB1 high*	*mean expression for AIB1 low*	*Fold (AIB high/AIB low)*	*Up (+) or down (-) regulated by AIB1*	*rank of P value of the randomized variance test*	*P value of the randomized variance test*
sialyltransferase 8D (alpha-2, 8-polysialytransferase)	206925_at	109.627	318.894	0.344	-	8	0.0000721
sialyltransferase	204542_at	230.423	442.079	0.521	-	59	0.0007661
palmitoyl-protein thioesterase 1 (ceroid-lipofuscinosis, neuronal 1, infantile)	200975_at	2632.758	5182.401	0.508	-	66	0.0008407
spastic ataxia of Charlevoix-Saguenay (sacsin)	213262_at	46.648	23.442	1.99	+	79	0.0011206
proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional protease 7)	209040_s_at	213.011	708.401	0.301	-	118	0.0023201

Table 1g

"secreted factors"	*Probe set*	*mean expression for AIB1 high*	*mean expression for AIB1 low*	*Fold (AIB high/AIB low)*	*Up (+) or down (-) regulated by AIB1*	*rank of P value of the randomized variance test*	*P value of the randomized variance test*
chromagranin A (parathyroid	204697_s_at	598.087	242.007	2.471	+	9	0.0000778
chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	203666_at	551.907	258.868	2.132	+	10	0.000082
bone morphogenetic protein 7 (osteogenic protein 1)	211259_s_at	307.814	140.98	2.183	+	18	0.0001298
inhibin alpha	210141_s_at	475.26	212.213	2.24	+	85	0.0012506
inhibin, beta B (activin AB neta polypeptide)	205258_at	1782.915	1014.699	1.757	+	77	0.0011023
hemoglobin alpha 2	209458_x_at	890.865	398.769	2.234	+	124	0.002482

Table 1h

"transport"	*Probe set*	*mean expression for AIB1 high*	*mean expression for AIB1 low*	*Fold (AIB high/AIB low)*	*Up (+) or down (-) regulated by AIB1*	*rank of P value of the randomized variance test*	*P value of the randomized variance test*
PDZ-1 domain containing 1	205380_at	699.117	211.344	3.308	+	25	0.0001706
karyopherin alpha 1 (importin alpha 5)	202056_at	424.574	181.884	2.334	+	27	0.0001824
karyopherin alpha 1 (importin alpha 5)	202055_at	959.842	391.313	2.453	+	28	0.0001887
potassium channel, subfamily K, member 5	219615_s_at	566.977	259.781	2.183	+	36	0.0003647
solute carrier family 25 (mitochondrial carrier; ornithine transporter) member 15	218653_at	823.559	404.301	2.037	+	50	0.0005662
sorting nexin 5	217792_at	1105.939	615.404	1.797	+	68	0.0008456
translocase of outer mitochonodrial membrane 22 homolog (yeast)	217960_s_at	315.36	675.567	0.467	-	69	0.0008724
MRS2-like, magnesium homeostasis factor (S. cerevisiae)	218536_at	433.963	241.151	1.8	+	91	0.0014565
aldehyde oxidase 1	205082_s_at	92.882	316.055	0.294	-	104	0.001889
acidic (leucine-rich) nuclear phosphoprotein 32 family, member A	201051_at	2169.058	1210.09	1.792	+	110	0.0020493

Table 1i

"transcription"	*Probe set*	*mean expression for AIB1 high*	*mean expression for AIB1 low*	*Fold (AIB high/AIB low)*	*Up (+) or down (-) regulated by AIB1*	*rank of P value of the randomized variance test*	*P value of the randomized variance test*
***AIB1*	*209062_x_at*	*1682.874*	*726.757*	*2.316*	+	*4*	*0.0000377*
Pirin	207469_s_at	198.894	669.774	0.297	-	15	0.0001229
NCOA3	211352_s_at	2164.567	1043.125	2.075	+	16	0.0001234
PHD finger protein 10	221787_at	936.315	458.354	2.043	+	26	0.0001719
polymerase (RNA) II (DNA-directed) polypeptide D	214144_at	146.593	254.863	0.575	-	41	0.0004117
histone deacetylase 1	201209_at	1258.795	2076.041	0.606	-	82	0.00117
Mads box transcription factor 2D myocyte enhancer factor 2D (MEF2D)	203003_at	71.257	27.906	2.553	+	96	0.0015729
F-box and leucine-rich repeat protein 11	208988_at	544.302	942.391	0.578	-	119	0.0023575

Table 1j

"miscellaneous"	*Probe set*	*mean expression for AIB1 high*	*mean expression for AIB1 low*	*Fold (AIB high/AIB low)*	*Up (+) or down (-) regulated by AIB1*	*rank of P value of the randomized variance test*	*P value of the randomized variance test*	*putative function*
ATAXIN1 (atxn1)	203231_s_at	78.312	276.067	0.284	-	14	0.000118	RNA binding; location nucleus
hypothetical protein FLJ10842	218568_at	399.582	190.925	2.093	+	19	0.0001303	diacylgycerol kinase activity
hypothetical protein MGC2963	221255_s_at	836.521	1710.86	0.489	-	24	0.000166	
MHC class I polypeptide-related sequence	206247_at	667.084	308.834	2.16	+	29	0.0001928	protein binding
pleckstrin homology domain containing, family B (evectins) member 2	201411_s_at	517.485	1117.563	0.463	-	34	0.000316	
S100 calcium binding protein A14	218677_at	1140.565	2211.092	0.516	-	39	0.0003937	calcium binding
olfactomedin 1	213131_at	1061.422	570.195	1.862	+	40	0.0004057	latrotoxin receptor; development neurogenesis
butyrophilin, subfamily 3, member A3	38241_at	67.413	177.872	0.379	-	42	0.0004601	immunoglobulin-like
WW domain binding protein 11	217821_s_at	293.572	492.855	0.596	-	43	0.0004607	ssDNA binding; colocalizes with mRNA splicing
synaptotagmin I	203998_s_at	133.708	249.236	0.536	-	45	0.0004762	integrin membrane; Ca sensor
DICER-1	213229_at	2307.863	1114.54	2.071	+	47	0.000515	
zinc finger, DHHC domain containing 3	218078_s_at	515.903	307.607	1.677	+	53	0.0006223	
trophoblast-derived noncoading RNA	214657_s_at	378.673	934.216	0.405	-	55	0.0006377	
KIAA0657 protein	212776_s_at	1433.679	769.539	1.863	+	56	0.0007141	
lin-7 homology A (C. elegans)	206440_at	232.047	447.247	0.519	-	60	0.000769	protein binding; exocytosis
hypothetical protein FLJ21031	220033_at	123.138	200.674	0.614	-	62	0.0007865	
zing finger protein 227	217403_s_at	70.891	135.505	0.523	-	63	0.0008073	
golgi autoantigen, golgin subfamily a, 2	204384_at	250.411	536.387	0.467	-	64	0.0008243	component of golgi apparatus
abhydrolase domain containing 3	213017_at	300.266	612.578	0.49	-	65	0.0008298	catalytic activity
golgi phosphoprotein 2	217771_at	334.384	162.16	2.062	+	71	0.0009642	
solute carrier family 9 (sodium/hydrogen exchange), isoform 3 regulatory factor 1	201349_at	5586.867	3087.018	1.81	+	73	0.0009937	protein binding; Wnt receptor signaling
sarcoma antigen	220793_at	180.675	351.721	0.514	-	74	0.0010088	tumor expressed antigen
toll-like receptor 3	206271_at	103.563	186.645	0.555	-	75	0.0010269	dsRNA binding; immunity response
damage-specific DNA binding protein 2, ‹	203409_at	317.875	547.097	0.581	-	78	0.0011139	damaged DNA repair; nucleotide excision repair
MHC class I region ORF	206082_at	178.538	377.635	0.473	-	83	0.0011727	defense response
phosphodiesterase 4A, cAMP-specific (ph	204735_at	212.51	121.629	1.747	+	84	0.001249	cAMP specfic phosphodiesterase; signal transduction
TP53 target gene 1	209917_s_at	278.896	739.962	0.377	-	86	0.0012653	response to DNA damage; signal transduction
spermatogenesis associated 2	204434_at	402.499	247.275	1.628	+	87	0.0012892	spermatogenesis; unknown function
hypothetical protein LOC90333	214751_at	132.651	281.339	0.471	-	90	0.0014372	
DKFZP586O0120 protein	201863_at	1066.931	2047.963	0.521	-	95	0.0015545	
Mouse Mammary Tumor Virus Receptor l	212484_at	1121.985	613.647	1.828	+	97	0.0016537	
KIAA0657 protein	212775_at	1599.555	879.879	1.818	+	100	0.001845	
Calmodulin regulated spectrin-associated	212712_at	550.83	331.554	1.661	+	102	0.0018503	
KIAA0515	212069_s_at	807.408	445.35	1.813	+	105	0.0019455	
golgi autoantigen, golgin subfamily a, 2	35436_at	467.131	849.159	0.55	-	109	0.0020245	
frequently rearranged in advanced T cell l	209864_at	606.599	1012.928	0.599	-	115	0.0022226	
testis expressed sequence 27	218020_s_at	1470.697	941.449	1.562	+	122	0.0024394	
suppression of tumorigenicity 7	207871_s_at	128.766	226.667	0.568	-	123	0.0024562	
hemoglobin alpha 2	209458_x_at	890.865	398.769	2.234	+	124	0.002482	

Table 1. cDNA microarray results of genes that are significantly altered by AIB1 siRNA treatment of MCF-7 cells.

*Two sample t tests were carried out using the randomized variance model. 124 genes were found to be significantly different between AIB1 high (control transfected) and AIB1 low (AIB1 siRNA transfected) cells at a $p \leq 0.0025$. The 124 genes were ranked according to p value. The 124 genes were separated into tables based on function and process as defined by the National Center for Biotechnology Information (NCBI) Entrez Gene database. Genes were categorized in the following classes: apoptosis (table 1a), cell cycle/proliferation (table 1b), cytoskeleton modifying (table 1c), GTP binding (table 1d), metabolism/biosynthesis (table 1e), protein modification (table 1f), secreted factors (table 1g), transport (table 1h), transcription (table 1i), and miscellaneous (table 1j). **genes selected for further analysis.*

Figure 1

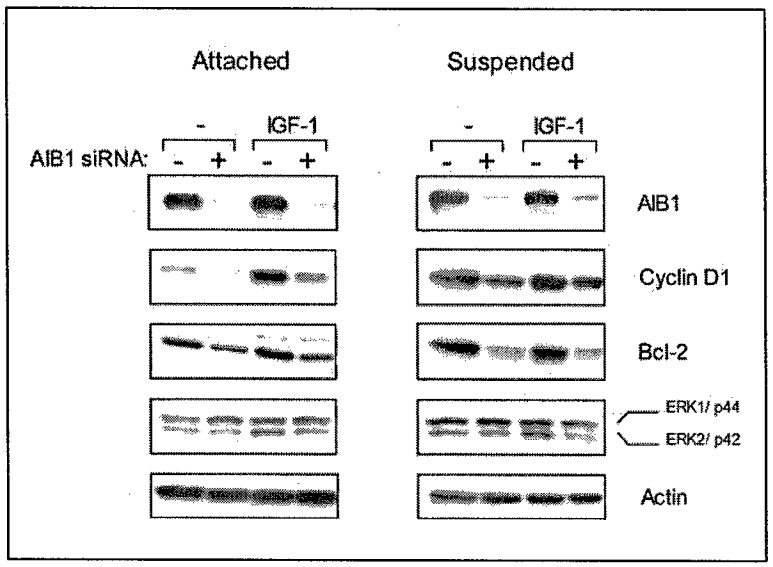

Figure 1. Changes in protein expression of critical genes involved in proliferation, cell cycle and apoptosis after AIB1 siRNA treatment. *For attached cells, estrogen stripped MCF-7 cells were transfected with control or AIB1 siRNA and treated with or without IGF-1 in the presence of 1% CCS. Whole cell lysates were harvested after 48 hours and analyzed by western blot. In suspension growth conditions, cells were transfected for 18-24 hours with siRNA and replated in poly-HEMA (10 μg/ml) coated dishes for another 24 hours before whole cell lysates were harvested and analyzed by western blot. Actin levels were analyzed to determine equal loading of samples. Blots are representative results from three independent experiments.*

Status of Task 2: Experiments described in the approved grant application are still ongoing.

KEY RESEARCH ACCOMPLISHMENTS

- cDNA microarray analysis is an effective way to determine the simultaneous changes in gene expression changes of over 30,000 genes.
- Genes that are critical for cell cycle regulation, anoikis, and apoptosis, such as Cyclin D1, Bcl-2, and ERK2 are regulated by AIB1 and Δexon3 AIB1 isoform.
- AIB1 and Δexon3 AIB1 isoform are necessary for the IGF-1 upregulation of Cyclin D1, Bcl-2, and ERK2.

REPORTABLE OUTCOMES

Manuscripts in preparation:

1. **Oh, A.S.**, List, H.J., Reiter, R., Mani, A., Zhang, Y., Gehan, E., Wellstein, A., and Riegel, A.T. The Nuclear Receptor Coactivator AIB1 mediates Insulin-Like Growth Factor-induced Phenotypic Changes in Human Breast Cancer Cells.
2. Tilli, M.T., Reiter, R., **Oh, A.S.**, Henke, R.T., McDonnell, K, Gallicano, G.I., Furth, P.A., and Riegel, A.T. Overexpression of an N-Terminally Truncated Isoform of the Nuclear Receptor Coactivator Amplified in Breast Cancer 1 Leads to Altered Proliferation of Mammary Epithelial cells in Transgenic Mice.

Doctoral Thesis in preparation:

- Role of Nuclear Receptor Coactivator AIB1 in Insulin-Like Growth Factor-1 Signaling in Human Breast Cancer. Doctoral dissertation of **Annabell S. Oh, B.S.** from the Department

of Tumor Biology, Georgetown University.

CONCLUSIONS

The majority of experiments in **Task 1** of the approved grant involving the co-activating effect of AIB1 and Δexon3 AIB1 isoform on EGF and IGF-1 signaling in a breast cancer model has been completed and is continuing to yield positive results. AIB1 and Δexon3 AIB1 isoform have a role in regulating the basal and IGF-1 induced expression of Cyclin D1, Bcl-2 and ERK2. It would be of high interest to determine if the regulation of these genes results in effects on apoptosis and cell cycle progression. IGF-1 and EGF signaling activate two major downstream signaling pathways that lead to anti-apoptosis and proliferation – mitogen activated protein kinase (MAPK) signaling and phosphotide-inositol triphosphate kinase (PI-3K) signaling. It would be interesting to determine if AIB1 and Δexon3 AIB1 isoform are required for maintaining or enhancing signaling through these major pathways.

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